THE EFFECTS OF HEAT ON THE DEVELOPMENT OF THE TOAD'S EGG.

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An extended series of experiments made by Hertwig (1–4) prove that the maximum temperature at which the eggs of the frog will develop normally differs for different species. His experiments also show that eggs in the cleavage stages can withstand a higher temperature than can unsegmented eggs. These results have a bearing on the general problem of adaptation ; for it may be possible to show, after more species have been studied, that the maximum temperature which the eggs of amphibians can endure without injury and also the temperature most favorable for their development depend, to a certain extent at least, on the time of year at which the eggs are deposited.

Material and Method.

The eggs of the common toad, *Bufo lentiginosus*, were used in making all of the experiments recorded in the present paper. After natural fertilization, the eggs were brought into the laboratory where the temperature varied from 18 to 21° C. Control sets of eggs from each lot used for the experiments, developing at the room temperature, all became perfectly normal embryos, and some of them were kept until metamorphosis.

In making the experiments, small dishes containing about 80 c.c. of spring water were placed in the drying chamber of a large water-bath, and after the water had become heated, from 50 to 75 eggs were quickly transferred into it and left a given length of time. The temperature to which the eggs were being subjected could readily be told from a thermometer that projected into the chamber through a small opening in the top. Great care was taken to keep the temperature of the chamber as constant as possible during the course of the experiments, and in no case did it vary more than two degrees. After the eggs were removed from the chamber, they were put into fresh water at room

temperature and their later development compared with that of the eggs in the control set.

II. Experiments on Unsegmented Eggs.

Experiment 1. — On April 16, twenty-five unsegmented eggs were subjected to a temperature of 28–30° C. for two and one-half hours. When removed from the chamber, all of the eggs were in the 16-cell stage, while in the control set, developing at room temperature, the eggs had only reached the 4–8-cell stage. The immediate effect of the higher temperature, therefore, was to increase the rate of development. This result agrees fully with that obtained by Hertwig in many of his temperature experiments on the frog's egg. The later development of the eggs in this series appeared to be perfectly normal, and it took place at about the same rate as in the eggs of the control set.

Experiment 2. — A number of eggs that had not yet segmented were put into water at a temperature of 30–32° C. on April 17. Part of the eggs were removed at the expiration of three quarters of an hour, and when examined they were all found to be segmenting. In a few cases the first cleavage plane had nearly cut through the yolk portion of the egg and the second furrow was appearing. In the control set of eggs, the first cleavage plane was just coming in at this time, so that, in this experiment also, the early development became more rapid as an immediate result of exposing the eggs to a higher temperature. All of these eggs developed into normal embryos.

Some of the eggs of the above lot remained in the heated chamber for one hour. The second cleavage plane had appeared in all of the eggs when they were removed to room temperature. Later segmentation was normal, and on the following day the dorsal lip of the blastopore appeared in all of the eggs at about the same time that it formed in the eggs of the control set. On April 19, many of the eggs were dead; some were in the early gastrula stages, and some showed traces of the medullary folds. Of the seven embryos alive on April 20, three were abnormal, having a large yolk plug exposed at the posterior end of the body; the other four embryos were normal and were kept for several weeks.

The remaining eggs of this lot were kept at the temperature of 30–32° C. for one and one-half hours. At the end of this time they were in the 16-cell stage, while the eggs of the control set were only in the 2–4-cell stage. Later segmentation of these eggs seemed to be normal, and on April 18 the dorsal lip of the blastopore appeared in a very few of them. On the morning of April 19 most of the eggs were dead, and not one of them, when examined, was found to have gastrulated. In the eggs still living the blastopore was closing in, but development was much slower than that of the eggs of the control set in which, at this time, the blastopore had already closed and the medullary folds were forming. All of the eggs were dead on the morning of April 20, and in no case was gastrulation entirely completed.

In these last two lots of eggs the injurious effects of heat were not apparent during the segmentation stages and only manifested themselves when the eggs were ready to gastrulate. Early development was accelerated; but later development lagged behind, or, at most, was equal to that of the eggs in the control set.

Experiment 3. — A number of unsegmented eggs were exposed to a temperature of 32° C. for two hours on April 22, and when removed they were in the 16-cell stage. In this lot of eggs the later cleavage was very abnormal as the upper hemisphere divided into a number of small cells, while the lower part of the egg segmented only a few times and, consequently, was composed of a small number of very large cells. Cleavage lines were very distinct in the upper part of the egg; but it was almost impossible to make out the boundaries of the yolk cells. None of the eggs in this set gastrulated and all of them were dead by April 24.

Experiment 4. — On the morning of April 16, a small lot of eggs was subjected to a temperature of 32–33° C. for one-half of an hour. The eggs had not segmented when they were put into cooler water, but in every case the first furrow appeared in about fifteen minutes. In the control set, the first cleavage plane came in about half an hour later than it did in the eggs used for the experiment. All of the eggs of this set developed

normally, and sections made of later embryos showed them to be no different from the embryos of the control set.

Experiment 5.— A bunch of about seventy-five unsegmented eggs was put into water heated to a temperature of 34–35° C. on April 16. Part of the eggs were removed at the end of half an hour and a few of them at once began to segment. None of the cleavage planes, with the exception of the first, came in normally, and in no case did any of them cut through the entire egg. Part of a section of one of these eggs is shown in Fig. 1. All of the cleavage planes are seen to be parallel and to extend but a short distance through the upper hemisphere of the egg. Development did not progress beyond this stage in any case, and the majority of the eggs never segmented although they appeared to be living several hours after they were brought into room temperature.

Some of the eggs of the above lot remained at the temperature of 34–35° C. for one hour. When put into cooler water and examined, a slight depression was found in the center of the upper hemisphere of a few of the eggs as if the first cleavage plane was about to appear in its normal position. This appearance, however, proved to be only a wrinkling of the surface as none of the eggs, when sectioned, showed any true cleavage planes.

The above experiments show that the unsegmented eggs of the toad can withstand a temperature of 32–33° C. for one-half of an hour and develop normally, while an exposure to this temperature for a longer period is very injurious and only a small per cent. of the eggs produced normal tadpoles. Exposure to a temperature of 34°, even for a short time, injures the eggs beyond the possibility of a recovery. The maximum temperature that the unsegmented egg can endure without injury is, therefore, 33° C. The optimum temperature, a term defined by Hertwig (3) as, "Die Temperatur bei welcher sich der Entwicklungsprocess bei allen Eiren mit der grössten Beschleunigung ohne eine auffällige Störung und Abweichung von der Norm vollzieht," for this egg is probably not far from 28° C., judging from the results obtained in experiments 1 and 2. In all cases in which the heat did not kill the eggs, development was accelerated at first, apparently with no injurious effects on the egg. In later stages,

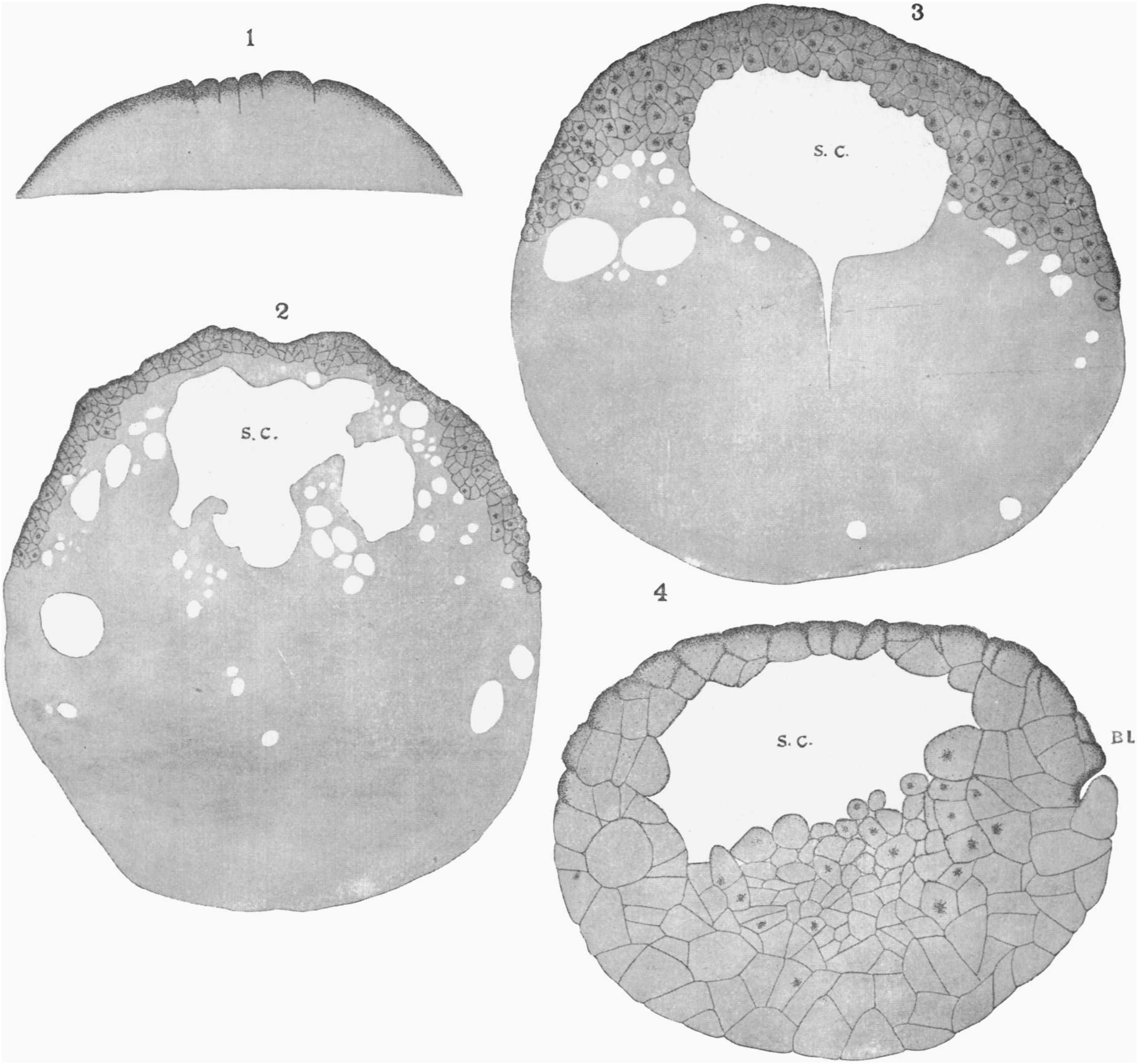

FIG. 1. Part of a section of an egg that was subjected to a temperature of 34–35°C. for one-half of an hour before cleavage began.

FIG. 2. A section of an egg that was exposed to a temperature of 35–36° C. for three quarters of an hour when it was in the two-cell stage. *S.C.*, segmentation cavity.

FIG. 3. A section of an egg that was subjected to a temperature of 33–35° C. for two hours after the first cleavage plane had appeared.

FIG. 4. A section of an egg that was subjected to a temperature of 31–33° C. for three and one-half hours when it was in the 32–64-cell stage of development. *Bl.* blastopore.

however, the eggs of the control sets appeared to be fully as far advanced in development as were the eggs that had been subjected to a higher temperature. Increase in the rate of development is, therefore, but the immediate effect of heat, and after the eggs are brought into a lower temperature they develop at the same, or a lower rate, than the eggs of the control set.

III. Experiments on Eggs in Early Cleavage Stages.

Experiment 6. — On April 17, a lot of about fifty eggs in the 2-cell stage of development was exposed to a temperature of 31–33° C. At the end of one and one-half hours, part of the eggs were removed. They were then in the 8–16-cell stage. The later development of these eggs was perfectly normal in every respect.

The rest of the eggs of this lot remained in the heated chamber for two hours. All of these eggs developed normally during the early cleavage and gastrulation stages; but later a few embryos were found with shortened medullary folds and a large yolk plug at the posterior end of the body. This form of abnormality is very common among embryos that have been injured by exposure to heat.

Experiment 7. — As the first cleavage plane was appearing, a lot of about fifty eggs was subjected to a temperature of 35–36° C. for three quarters of an hour. All of the eggs were segmenting in a very abnormal manner when they were transferred into water at the room temperature, and none of them ever gastrulated. Fig. 2 shows a median section through one of these eggs. With the exception of the layer of small cells bordering the outer surface of the upper hemisphere, the entire substance of the egg is seen to be unsegmented and to have a number of different sized vacuoles scattered through it. A large, irregularly shaped cavity fills the greater part of the upper hemisphere of the egg. This cavity is much larger than the segmentation cavity in a normally segmenting egg, and it appears to be formed of the true segmentation cavity and several large vacuoles which have come to open into it.

Experiment 8. — On April 22, a lot of eggs in the 2-cell stage was exposed to a temperature of 35–36° C. for one hour. When

removed from the chamber the eggs were in the 8-cell stage, but development stopped at this point and all of the eggs were dead inside of twenty-four hours.

Experiment 9. — In this experiment, eggs in the 2- and in the 4-cell stages of development, were subjected to a temperature of 33–35° C. for a period of two hours. At the end of this time the eggs were segmenting very irregularly in the upper hemisphere and no cleavage planes were visible in the yolk portion of the egg. A section through one of these eggs (Fig. 3) shows the entire upper hemisphere divided into a mass of small cells containing a considerable amount of pigment which is, for the most part, collected in the middle of the cell around the nucleus. The first cleavage plane has cut only partially through the yolk portion of the egg, as its progress was evidently stopped at the beginning of the experiment. There are no nuclei in the yolk portion of the egg, and the many vacuoles show the injurious effects of the heat. The mass of small cells in the upper hemisphere forms a sort of cap on the unsegmented yolk and make it appear as if the segmentation of the egg was meroblastic. This same sort of abnormal cleavage has also been obtained by Hertwig (1, 2).

According to the experiments in this series, eggs in the early cleavage stages can endure exposure to a temperature of 31–33° C. for a longer period than can the unsegmented egg; yet they are permanently injured by even a short immersion in water at a temperature of 35°. The maximum temperature for these eggs, therefore, is not greater than that for the unsegmented egg. Hertwig (4) has found that the maximum temperature for the eggs of *Rana fusca* in the 8-cell stage of development is 26–28°, which is 3–4° higher than that for the unsegmented egg.

IV. Experiments on Eggs in Late Segmentation and Early Gastrula Stages.

Experiment 10. — On April 18, fifty eggs in the 32–64-cell stage of development were kept at a temperature of 31–33° C. for two hours. Subsequently all of the eggs developed into normal embryos and at about the same rate as did the eggs of the control set.

Experiment 11. — Another set of fifty eggs from the same bunch as the eggs used in experiment 10, was subjected to a temperature of 31–33° C. for three hours. The late segmentation and early gastrulation stages of all of these eggs seemed to be perfectly normal. Two days after the experiment was made, 38 of the eggs were dead, the blastopore not having closed in any case. Of the remaining eggs four only were normal, the rest had a large yolk plug at the posterior end of the body.

Experiment 12. — Twenty-five eggs from the same lot as those used in the two preceding experiments remained in water at a temperature of 31–33° C. for three and one-half hours. Fifteen of the eggs died in the blastula stage. The blastopore appeared in the other ten eggs, but in many cases it was in an unusual position at the equator of the egg. When the dorsal lip of the blastopore was forming in these eggs, the circular blastopore was already beginning to close in the control set of eggs, therefore, in this instance, the heat retarded instead of increased the rate of development of the eggs. In none of the eggs of this set did the blastopore ever become circular, and all of the eggs were dead two days after the experiment was made.

Fig. 4 shows a section of one of these eggs preserved when the blastopore appeared in surface view as a short, straight line at the equatorial zone. The dorsal lip of the blastopore rarely, if ever, comes in as high up as the equator in eggs that are developing normally; but it sometimes occupies an unusual position in eggs that have been subjected to abnormal conditions. Morgan (5) has found the blastoporic rim above the equatorial zone in eggs of *Rana palustris* that have been subjected to intense cold. In Fig. 4 the archenteron appears as a shallow depression with its dorsal wall formed of heavily pigmented cells as is normally the case. The inner end of the archenteron, instead of turning up towards the black pole as it would do in a normal egg, here projects downward towards the yolk pole. The most interesting fact shown by the section is that the normal position of the large and of the small cells of the egg is completely reversed. In normally gastrulating eggs, the roof of the segmentation cavity is formed of two to three layers of small, pigmented cells, while the ventral wall is composed entirely of large

yolk cells that contain little, if any, pigment. In this egg, however, the upper wall of the segmentation cavity is made up of a single layer of heavily pigmented cells which are fully as large as any other cells in the egg. Below the segmentation cavity, a portion of the yolk is divided into a number of small cells, many of which contain pigment massed around the nucleus. Some of these cells are rounded and seem to lie free in the segmentation cavity, an appearance also noted by Hertwig (4) in eggs of *Rana fusca* that were exposed to a temperature of 29–35° C. after having reached about the 100-cell-stage of development.

Morgan has also noted the relatively large size of the cells in the upper hemisphere of gastrulating eggs of *Rana palustris* that had been subjected to cold. He suggests that this increase in the size of the cells "may be due in part to the absorption of water by the individual cells," and he adds that, "even if this is the case the cells are fewer in number than in a normal egg beginning to gastrulate." In the figure shown by Morgan, the cells of the lower hemisphere are all considerably larger than those of the upper hemisphere; the egg, therefore, must have been much more normal than the one from which Fig. 4 was drawn.

It is evident, in the case of the egg shown in Fig. 4, that the increased temperature did not injure the yolk region or retard its development as is usually the case in these experiments; on the contrary, it is the segmentation of the upper hemisphere that has been delayed, while the segmentation of the lower portion of the egg has continued. No egg in this set of experiments developed much beyond the stage represented by Fig. 4, and each of the ten eggs that were sectioned showed abnormalities of the same general character.

Experiment 13. — On April 26, about seventy-five eggs in the late blastula stage were subjected to a temperature of 33–35° C. A part of the eggs were removed at the end of one and one-half hours and they all developed into normal embryos.

A second portion of the eggs was exposed to this temperature for two and one-half hours. All of these eggs developed into normal embryos, although somewhat more slowly than did those of the control set.

A third part of the eggs remained at the temperature of 33–35° for three and one-half hours. These eggs were all dead when removed from the influence of the heat.

Experiment 14. — A number of eggs in the blastula stage were exposed to a temperature of 36–37° C. on April 26. Some of the eggs were removed from the chamber at the end of one-half of an hour. The eggs did not appear to be injured in any way by the experiment and all developed normally.

A second portion of the eggs from the above lot remained at this temperature of 36–37° C. for three quarters of an hour. All of the eggs gastrulated normally, but about half of them died before the blastopore closed. When sectioned these eggs showed no abnormalities. The rest of the eggs became normal embryos, although developing very slowly. The medullary folds had closed in the eggs of the control set when they were only beginning to unite in the eggs that had been subjected to the increased temperature.

The remaining eggs of this lot were removed to room temperature at the end of one hour. Although the eggs did not appear to be dead when they were examined, they did not gastrulate and none of them were alive the day following the experiment.

Experiment 15. — Twenty eggs in late segmentation stages were subjected to a temperature of 40–42° C. for one quarter of an hour. Development was at once stopped by the heat, and all of the eggs were killed.

Experiment 16. — When the dorsal lip of the blastopore was just appearing, a lot of about twenty eggs was put into water at a temperature of 33–35° C. and left there for three hours. All of the eggs continued to develop somewhat more slowly than the eggs of the control set and all became normal embryos.

Experiment 17. — On April 24, a lot of eggs in early gastrulation stages was kept at a temperature of 35–37° C. for one hour. In all of the eggs the lateral and ventral lips of the blastopore formed in the normal manner, but development stopped at this point and the eggs died. No abnormalities were detected when sections were made of several of these eggs.

Experiment 18.—Eggs in early gastrulation stages were exposed to a temperature of 37–38° C. on April 24. A part of the eggs were removed at the end of one quarter of an hour. None of these eggs seemed to be injured in any way by the high degree of heat to which they had been subjected and all developed, somewhat slowly, into normal embryos. The rest of the eggs in this lot remained at the temperature of 37–38° C. for one hour. They were all dead when removed to room temperature.

The results of the experiments in this series show that eggs in the 32–64-cell stage cannot withstand a temperature of 31–33° C. for a much longer period than can eggs that have just begun to segment. The maximum temperature to which eggs can be subjected without injury is practically the same for unsegmented eggs and for those in early cleavage stages, although eggs in the later stages can remain at this temperature for a somewhat longer period and still develop normally.

Eggs in late cleavage stages have a much greater power to withstand high temperature than have eggs in the earlier stages of development, as they will develop normally after exposure to a temperature of 36–37° C. for one-half of an hour. The maximum degree of heat that can be endured without injury is still higher for eggs in the gastrula stages, as they become normal embryos after being subjected to a temperature of 37–38° C. for one quarter of an hour.

The experiments described above are summarized in the following table. The number of the experiment is given in the first column; the condition of the eggs when the experiment was begun in the second column; the temperature to which the eggs were subjected in the third column; followed in the next two columns by the duration of the experiment and a brief statement of the results.

The results of these experiments are very similar to those obtained by Hertwig (1–4) in his study of the effects of heat on the development of the eggs of various species of frogs; and the abnormalities produced resemble, in many respects, those which Hertwig has described and figured. When the unsegmented eggs of *Bufo lentiginosus* are subjected to a temperature that

TABLE I.

No. of Exp.	Condition.	Temperature.	Time.	Result.
1	unsegmented.	28–30° C.	2½ hrs.	Normal development.
2	"	30–32	¾ "	Normal development.
2	"	"	1 "	Four eggs developed normally; the rest died or became abnormal.
2	"	"	2½ "	Most of the eggs died in the blastula stage; a few gastrulated but did not develop further.
3	"	32	2 "	All died in the blastula stage.
4	"	32–33	½ "	Normal development.
5	"	34–35	½ "	Irregular cleavage, no gastrulation.
5	"	"	1 "	Eggs killed.
6	2 cell.	31–33	1½ "	Normal development.
6	"	"	2 "	Most of the eggs developed normally.
7	"	35–36	¾ "	Abnormal cleavage, no gastrulation.
8	"	"	1 "	Development stopped at the eight-cell stage.
9	2–4 cell.	33–35	2 "	Abnormal cleavage, no gastrulation.
10	32–64 cell.	31–33	2 "	Normal development.
11	"	"	3 "	Four normal embryos; the rest of the eggs died or became very abnormal.
12	"	"	3½ "	All of the eggs became abnormal, none of them developed into tadpoles.
13	Late seg.	33–35	1½ "	Normal development.
13	"	"	2½ "	Normal development.
13	"	"	3½ "	Eggs killed.
14	"	36–37	½ "	Normal development.
14	"	"	¾ "	A few of the eggs developed normally, most of them died in the gastrula stage.
14	"	"	1 "	Eggs killed.
15	"	40–42	¼ "	Eggs killed.
16	Early gastrula.	33–35	3 "	Normal development.
17	"	35–37	1 "	Development stopped when the blastopore was closing in.
18	"	37–38	¼ "	Normal development.
18	"	"	1 "	Eggs killed.

stops their development before gastrulation begins, sections of the eggs show, in many cases, that the greatest injury has been produced in the yolk portion of the egg which is frequently vacuolated and not segmented although the upper part of the egg has divided into a large number of small cells. Hertwig has noticed the same phenomenon in some of his experiments, and in explanation he states as follows: "Dass Froscheier bei erhöhter Temperatur zunächst partiell geschädigt werden und eventuell absterben, ist offenbar auf die verschiedene Organisation der animalen und vegetativen Hälfte der Dotterkugel zurück-

zuführen. Die animale Hälfte der Dotterkugel ist reicher an Protoplasma und steht in höherem Masse unter der Herrschaft des Zellkerns. Unter der normalen Wechselwirkung von Protoplasma und Kern können aber Schäden, welche eine Zelle erlitten hat, wie durch verschiedene Experimente festgestellt worden ist, wieder rückgängig gemacht werden. In dieser Beziehung findet sich die vegetative Hälfte der Eikugel unter ungünstigeren Bedingungen. Denn hier ist das Protoplasma nicht nur spärlicher zwischen den Dotterplättchen vertheilt, sondern ist auch am ungethielten Ei mehr dem Einfluss des Zellkerns, der in der animalen Hälfte liegt, entrückt; später, nach Ablauf der ersten Furchungsstadien sind die Theilstücke vielmals grösser als die aus der animalen Eihälfte entstehenden Zellen."

When the injurious effects of the heat are not manifested until the eggs gastrulate, Hertwig (3) finds, in *Rana fusca*, that the abnormalities produced are of two sorts: First, those with a large yolk plug in the posterior region; second, those with deformed heads. In all of my experiments on *Bufo*, the abnormal tadpoles, with but very few exceptions, were of the first sort described by Hertwig. In some cases the development of the eggs stopped when the medullary folds were forming and a large yolk plug was found in the mid-dorsal region; in three cases only was the defect in the anterior part of the embryo. My results are more in accord with Hertwig's experiments on *Rana esculenta* than with those on *Rana fusca*, as in his experiments on the former species he obtained a much smaller number of spina bifida embryos than of those with a large yolk plug at the posterior end of the body.

Hertwig (4) finds that the optimum temperature for the devel opment of *Rana fusca* is 20° C. for the unsegmented egg, and that this optimum rises gradually to 24° C. for eggs in later stages of development. He adds: "Offenbar hängt diese Erscheinung damit zusammen, dass mit der Vermehung der Zellen die Kernsubstanz im Verhältniss zum Protoplasma immer mehr zunimmt und dass so das Protoplasma in höheren Masse ihrem Einfluss unterworfen ist." The optimum temperature for the unsegmented egg of *Bufo lentiginosus* is undoubtedly higher than that for *Rana fusca*, and it is probably somewhere near 28°

C. This optimum in increased 2–3° for eggs in later stages of development.

In another set of experiments on *Rana fusca*, Hertwig (4) finds that the maximum temperature to which the unsegmented eggs can be subjected without suffering any injury is 23–24° C., while this maximum is increased to 30° C. for eggs in the late segmentation stages. The maximum temperature for unsegmented eggs of *Rana esculenta* Hertwig finds to be 33° C. This is also the maximum temperature I have found for unsegmented eggs of the toad, although eggs in the blastula stage can endure a temperature of 38° C. for a very short time.

Morgan has noted that the blastula stages of *Rana palustris* can endure extreme cold much better than can eggs in the 2–4-cell stages, and he also finds that the eggs of *Rana temporaria* which are laid very early in the spring, can survive the tempera- of freezing water for several days. This temperature would very soon kill eggs of *Rana palustris* which are deposited much later than are the eggs of *Rana temporaria.*

While the eggs of all of these species of *Anura* can withstand a wide range of temperature without injury, there appears to be an adaptation to temperature corresponding to the different periods at which the eggs are deposited. *Rana fusca* and *Rana temporaria* lay their eggs very early in the spring when the water is often at the freezing point; and the eggs of these two species can stand cold much better than can the eggs of *Rana palustris* and *Rana esculenta* which are laid considerably later. Although the eggs of *Bufo lentiginosus* are laid but little later than are those of *Rana palustris*, they are usually deposited in shallow pools of water exposed to the direct rays of the sun. They must, therefore, often be subjected to a comparatively high degree of heat during the course of their development.

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BRYN MAWR, PA., April 24, 1903.

BIBLIOGRAPHY.

1. **Hertwig, O.**
'94 Über den Einfluss äusserer Bedingungen auf die Entwickelung des Froscheies. Sitzber. der Kgl. Preuss. Akad. der Wiss. Phys.-math. Abth., Bd. XVII., 1894.

2. **Hertwig, O.**
'96 Über den Einfluss verschiedener Temperaturen auf die Entwickelung der Froscheier. Sitzber. der Kgl. Preuss. Akad. der Wiss. Phys.-math. Abth., Bd. XIX., 1896.

3. **Hertwig, O.**
'97 Ueber den Einfluss der Temperatur auf die Entwicklung von Rana fusca und Rana esculenta. Archiv f. mikr. Anat., Bd. LI., 1897.

4. **Hertwig, O.**
'99 Ueber das Temperaturmaximum bei der Entwicklung der Eier von Rana fusca. Cinquantenaire Soc. Biol. Paris, 1899.

5. **Morgan, T. H.**
'02 The Relation between Normal and Abnormal Development of the Embryo of the Frog, as Determined by Injury to the Yolk-Portion of the Egg. Archiv f. Entwickelungsmech., Bd. XV., 1902.